Prevalence And Chemotherapy Of Canine Giardiasis In Khartoum State, Sudan

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A Thesis submitted to the University of Kharoum in partial fulfillment of the requirements of the Degree of Master of Vetrinary Science M.V.SC.))

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Dedication

To my lovely family,
And all those who made this work possible

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Acknowledgements

I thank and praise, the merciful, the beneficent, the Almighty God for his guidence through out the period of the study.

I wish to express my gratitude and sincere thankfull to my supervisor Dr. EL Awad Mohamed EL Hassan, Department of parasitology Faculty of Veterinary Medicine. Whose guidance, criticism, encouragement and interest made this study possible. He patiently reviewed my numerous thesis drafts, made constructive and educative comments, and showed concern for any problem I encountered in the course of my study.

My special thanks to Dr. Ali Ahamed Hassan, head of Police Dog Adminstration for his permission to carry out this work in his department and for his continuos encouragement, generous help and support. And Dr.Abdaeen Mohamed Abdaeen, Dr. Khalid Mohamed Abdrahman, Dr. Amar Mohamed Albager and the staff of Police Dog Adminstration for their generous help and support.

I also acknowledge, with gratitude, and help received from Dr. Shawgi M. Hassan head department of parasitology, Faculty of Veterinary Medicine, University of Khartoum concerning the statistical analysis. For staff in department of parasitologe, Faculty of Vetrinary Medicine. For their valuable assistance.

Deepest thanks to my colleague Sara Musa, Mohamed Said, Ibrhim Adam Hassan for their help and encouragement.

Abstract

Giardia infection is widely distributed and data concerning its prevalence in dogs were reported from many parts of the world. Unfortunatly, in Sudan reports on canine giardiasis is lacking however the disease is actual problem to the owners and in. Reinfection after treatment is common feature.

In a survey conducted during the period from January 2003-Jaunary 2004 in Khartoum state, prevalence of Giardia infection was determined. Tow Hundered and eleven dogs were examined by faecal examination and an overall prevalence of positive dogs was 69%. Although all age groups were found susceptible to infection, young dogs showed the higher prevalence rate. 72.2% was detected among those less than one year old, while that of those between 1-3 years and those over 3 years was 61.3% and 63.7% respectively. The disease was found to prevail throughout the year, a prevalence of (71.9%) was detected during the winter, while a prevalence of (68.8%) was found during summer and a prevalence of (68.8%) was detected during autumn. 72.2% of positive animal were male and 64.4% were females.

In this study an experiment was performed to evaluate three diagnostic tools of giardiasis (direct faecal examination, zinc sulphate centrifugal floatation and ELISA) and to study the efficacy of some chemotherapeutic agents. Zinc sulphate centrifugal floatation was found more suitable for diagnosis Giardia infection in dogs. Chemotherapy trail revealed that fenbendazole is ceasing cysts shedding in infected dogs compared to metronidazole and the combination of the above mentioned drugs.

INTRODUCTION

Van Leeuwenhoek was the first to report Giardia species in man in 1681. However, the discription of the species *Giardia lamblia* (*Giardia duodenalis*; *Giardia intestinalis*) is attributed to Vilem Lambl who described the parasite under the named *Cercomonas intestinalis*. Subsequently, during the 19th century, a large number of different cases of giardiasis were described from man and other animals.

Giardia lamblia is often pathogenic in individuals who have no previous experience of the infection. It can be the cause of diarrhea among foriegn travelers in the tropics and has an epidimic potential for acute diarrheal illness in non-endemic areas. However, under normal circumstances *Giardia lamblia* infection is usually asymptomatic particularly in countries where the infection is common.

Approximately 20 to 30% of inhibtants in developing countries in Asia and Africa are infected with *Giardia lamblia* (Oda, Kawabata and Uga, 2005). In the Sudan *Giardia lamblia* is commonly present in stools of adult patients who present with gastrointestinal symptoms at the outpatients clinics (Salih and Abdalla, 1977).

The importance of canine Giardiasis is brought about by its high prevalence (reaching 100% in some kennels), seriousness as a disease entity, and a possibility as a zoonosis (Leib and Zajac, 1999; Faubert, 2000; Itoh, Muraoka, Mikiko, and Itagaki, 2001; Keulen, Macechko, Wade, Schaff, Wallis, and Erlandsen, 2002; Stuart, Orr, Warburton, Jeyakanth, Pugh, Moris, Sarangi and Nichols, 2003). The disease is common among puppies, while in old dogs it is usually asymptomatic.

Different diagnostic techniques are used to detect infection with Giardia species ranging from conventional parasitological methods, such as direct faecal smear examination, and zinc sulphate centrifugal floatation; to sophisticated serological methods such as immunofluorecence, enzyme linked immunosorbent assay (ELISA), counter immuno electrophoresis and endoscopy.

Chemotherapy of the disease include adminstration of Metronidazole {(1-β-hydroxyethyl) -2-methyl-5-nitromidazole; Flagyl} (Mendelson, 1980), and Fenbedazole (Zajac, LaBranche, Donoghue, and Chu, 1998; Faubert, 2000), with varing efficacy. However, recurrence of infection is not uncommon.

In the Sudan information concerning prevalence of giardiasis in dogs is lacking. The diagnosis of the disease in dogs rely on direct faecal smear examination of sick cases brought to veterinary clinics and positive cases are usually treated with Metronidazole.

This study was designed to serve the following objectives: -

1- To evalute three diagnostic methods for the diagnosis of giardiasis in dogs, namely, direct faecal examination, zinc sulphate centrifugal floatation method and enzyme linked immunosorbent assay (ELISA).
2- To determine the prevalence of giardiasis in dogs in Khartoum state.
3- To compare drugs used for treatment of giardiasis metronidazole, fenbendazole and their combination.

CHAPTER ONE
REVIEW OF LITERATURE

1.1. Historical Background of Giardia

Giardia spp. are flagellated parasitic protozoa that live in the small intestine of dogs, cats, cattle, various rodents, rabbits and other mammals including man (Nash, Herrington, Losonsky and Levine, 1987; Ortega and Adam, 1997; Itoh, *et al*, 2001; Anderson, Brooks, Morrison, Smith, Martin, Benn, and Peregrine , 2004). Its first description was attributed to the microscopist Antony van Leeuwenhoek in 1681, in his own faeces (Baker, 1973). Vilem Lambl (1824 to 1895), a Czech physician, was credited with the discovery in 1859 of *Giardia lamblia* flagellate. Blanchard gave the name lamblia to the species in 1888 (Faubert, 2000). But there is no evidence that either Leeuwenhoek or Lambl saw or recognized the cyst of the organism. This was first noted by Graaci in 1879 and was thought to be a coccidian parasite. Then Gracci associated the cyst form with the flagellated trophozoite form (Kreier and Wakelin, 1998). Although it was the first protozoan described, the role of Giardia as a pathogenic organism was not recognized until the 1970s, after community outbreaks in USA and after the appearance of the disease in travellers returning from endemic regions. Prior to that time, the organism was thought to be a harmless commensal organism inhabiting the intestine (Cash, Johnston and Bhutani, 2001).

1.2. Giardia Taxonomy

Giardia species are flagellated protozoa, classified according to Soulsby (1982) as follows:

Class: Zoomastigophorea (Calkins, 1909)

Order: Diplomonadida (Brugerolle, 1975)

Family: Hexamitidae (Kent, 1880)

Genus: Giardia (Knustler, 1882)

Different species of Giardia are structurally very similar. It was customary to name the species according to their hosts, thus the species in dogs is called *Giardia canis*; in cattle *Giardia bovis*, and so on. Filic (1952) used the morphological structure of the median body of the trophozoite microtubular organelle to classify Giardia species into three groups namely: (i) the amphibian group (*Giardia agilis*), which has a long tear-drop shaped median body; (ii) the rodent and bird group (*Giardia muris*), which has two small, rounded median bodies; and (iii) the human group (*Giardia duodenalis, lamblia* or *intestinalis*), in which the single or double median bodies resemble the claw of a hammer. *Giardia duodenalis* have been described not only in humans but also in other mammals, birds, and reptiles (Amar, Dear, Pedraza-Diaz, Looker, and Linnane, Mclauchlin 2002).

Isoenzyme and DNA analysis indicates that *Giardia duodenalis* is heterogeneous. Isolates identified as infectious for humans are classified into two major groups designated as assemblages A and B. *Giardia duodenalis* assemblage A has been further divided into two groups, subgroup I and II. Assemblage A group I has also been detected in the faeces of livestock, cats, dogs, beavers or guinea pigs. However, assemblage A group II being confined only to humans. *Giardia duodenalis* assemblage B has a broad host range and has been recovered from dogs, beavers and rats. Other genotypes of *Giardia duodenalis* occur; and these are specific to their named hosts and are designated the dog, cat, hooved animals, rat and muskrat genotypes. However, application of genetic analysis to *Giardia duodenalis*

parasites from human is limited, and there is considerable uncertainty to the role of animals as reservoirs of human infection, as well as whether the zoonotic origins of this parasite reflected genetically (Amar, *et al*, 2002).

1.3. Morphology

1.3.1. Morphology of Trophozoite

The trophozoite is a pear-shaped bilaterally symmetrical organism. The anterior end is broad while the posterior is somewhat pointed. The dorsal side is convex and the ventral side is concave with a large sucking disk in the anterior half (Soulsby, 1982). Above the sucker there are two nuclei, and between the later lie basal bodies. The trophozoite usually measures from 10 -20µ long, 5 - 10µ broad, and 2 - 4µ thick (Figure 1) (Baker, 1973).

1. 3.2. Morphology of the Cyst

The cyst of *Giardia lamblia* is oval in shape, about 8 - 14µ long and 6 - 8µ wide. It contains (when mature) four nuclei grouped at one end. The remains of the median bodies, flagella and the anterior rim of the sucker form a rather confused collection of fibrils within the cyst (Figure 2) (Baker, 1973).

1.4. Host Range

Giardia species infect a wide range of hosts. Dogs, man, cattle, rodents, rabbits and other mammals can all be infected and there is considerable uncertainy as to the relative role of animals as reservoirs

Figure (1): Giardia trophozoite avaliable at www.ivis.org

Figure (2): Giardia cyst avalialble at www. ivis.org

of human infection (Faubert, 1988; Bemrick and Erlandsen, 1988; Faubert, 2000).

1.5. Life Cycle

The life cycle is well known and conventionally: involving a trophozoite (feeding stage) and a cyst (infective stage) (Ackers, 1980; Faubert, 1988; Bemrick, and Erlandsen, 1988). It is presumed that infection normally follows ingestion of cyst with contaminated water or food. The cyst develops within 30 minutes into two daughter trophozoites in the duodenum (Ackers, 1980). This is followed by excystation, which occurs as a result of exposure to the acidic gastric pH and pancryatic enzymes chymotrypsin and trypsin, producing two trophozoite (Ortega and Adam, 1997). The trophozoite attaches to the intestinal wall, and occasionally invade the bile duct (Faubert, 2000). They do not ingest solid food, nor do they appear to dissolve tissue cells; possibly they feed on the abundant secretions of mucus, which their presence stimulates its production, and on a varity of aminoacids, vitamins, and other substances, which are constantly passing in and out of the intestinal mucosal cells (Chandler and Read, 1980). The trophozoite divides by binary fission every 5 to 10 minutes. After millions, or even billions of organisms are produced, natural processes in the intestine flush some of the trophozoites to the large bowel or colon. Adverse conditions there cause the trophozoite to transform itself into a cyst, possibly as a result of exposure to bile salts or from the cholestrol starvation (Ortega and Adam, 1997). This process is called encystation and it is the organism′s protective mechanism that developed in preparation for introduction into the external environment. Trophozoites are not normally found in the stool, but

in cases of diarrhea dead ones may be present in considerable numbers (Chandler and Read, 1980). There is no sexual stage known to occur in Giardia life cycle (Backer, 1973).

1.6. Epidemiology

Giardia cysts are resistant to destruction in hypotonic solution such as water (Meyer, and Jarrol, 1980). The cysts are relatively resistant to disinfection with ultraviolet light (Ortega and Adam, 1997). The length of their survival in water varies greatly, according to the degree of temperature. At 8ºC they survive for more than two months, while at 21ºC they survive for about one month, and at 37ºC they can only last for 4 days (Meyer, and Jarrol, 1980). Giardia cysts in moist environment like sewage sludge may survive as long as three months (Knight, 1980; Oslon, Hannigan, Gaviller, and Fulton, 2001), and it is believed that they may survive longer beneath finger nails (Knight, 1980). The usual concentrations of the chloramines, mercuric chloride, and formalin used for cleaning water have no effect on the survival of the cyst (Mahmoud and Warren, 1975; Stuart, *et al*, 2003).

Human infections with this organism occur endemically worldwide and have been reported from the tropic to the arctic. Twenty to 30% of inhibtants of developing countries in Asia and Africa are infected with this parasite (Oda, *et al*, 2005). This variation in prevalence may be due to geographical location, differences in the definition of the source population, differences in the methods used to determine prevalence, inappropriate methods of faecal analysis, difficulty in identifying Giardia cysts, and the intermittent shedding of the parasite (Anderson, *et al*, 2004) presumably

reflecting the low level of hygiene in some areas (Meyer, and Jarrol,1980) and insufficient water treatment facilities (Ortega and Adam, 1997).

Giardia isolates belonging to genotypes A and B of *Giardia lamblia* are widespread in nature and can be found in humans, farm animals, pets and some wild animals. This strongly raises questions concerning the origin of giardiasis, in particular infection with genotypes A and B, which could be zoonotic. However, it is not clear whether animals act as a reservoir host for humans or vice versa (Kasprazc, and Pawlowski, 1989; Itoh, *et al*, 2001; Mochizuki, Hashimoto, and Ishida, 2001; Amar,*et al*, 2002; Jimenez-Cardoso, Eligio-Garcia, and Cortes-Campos, 2002; Keulen, *et al*, 2002; Stuart, *et al*, 2003)

The disease in dogs is widely distributed and data concerning its prevalence in these animals were reported from many parts of the world. In Belgrade, faecal samples were examined from each of 78 pet dogs (34 puppies of <9 months, 25 dogs of 9 - 18 months and 19 adult of > 18 months) (Nikolic, Kulisic, and Bojkovski, 1992). Giardia cysts were detected in three of the puppies; while diarrhea was common in all 34 puppies examined. Stool samples from 3 owners of the infected puppies and 11 of their family members were negative for Giardia. In the west Scotland one hundred faecal specimens randomly collected from various location within 7 puplic parks, were examined for the presence of Giardia cysts (Grimason, Smith, Parker, Jackson, Smith and Girdwod, 1993). An average of 11% of the samples contained Giardia cysts. Occurrence data from individual parks varied from 0 to 40%. In Denmark, faecal samples collected from 97 dogs and examined for giardiasis showed a prevalence of 20% in kennels and 17.1% in young dogs (lower than one year) (Hansen, Nielsen, Monrad and Vibe, 2000). In Spain, the incidence of giardiasis was

studied in the province of Granda, from February 1984 to March 1985. Faecal samples were collected from 175 children, 157 dogs and 34 rats. Results showed that rats were more often infected with *Giardia lamblia* (14.7%) than children (8.6%) or dogs (10.2%); and that dog less than 6 months of age were more frequently infected than dogs older than 6 months (Diaz, Verdejo, Campos, Manas and Lozano, 1987).

In Egypt, a study was carried out from April to December 1997 to investigate the occurrence of Giardia cysts in dogs, cats, rodents and their possible implication in infantile diarrhea. A total of 400 faecal samples were collected from stray dogs and cats, 300 from rats and mice, and 400 from children (1 to 10 years old) with diarrhea. The prevalence of Giardia cysts in the samples were 24% in dogs, 11% in cats, 19.66%in rats and mice, and 21% in children, no morphological differences were seen emphasizing the inter species transmision of Giardia and its public health importance (Khalifa, 1999).

In Australia, in one year study (April 1991 – May 1992) to determine the occurrence of parasitic infection in dogs Giardia species ranged from 2.3 - 14.2% of the parasitic fauna detected in these dogs (Johnston and Gasser 1993).

In Malaysia, examination of faecal samples from a residential housing state in Penang showed that 52 dogs (21.9%) were infected with Giardia (Rahman, 1990). In Japan, a total of 1035 household dogs were examined for the prescence of Giardia cysts in their faeces. Faecal samples from 151 (14.6%) dogs were positive for Giardia. The form of the organisms obtained from the 151 dogs was cysts (77.5%), trophozoites (9.9%), or both cysts and trophozoites (12.6%). Dogs kept indoors had a higher prevalence (18.5%) of Giardia than dogs kept outdoors (4.8%). Giardia infection was also more

prevalent in 1 - 6 months old puppies (21.7%). The prevalence of Giardia infection in dogs originally purchased from pet shops or breeding kennels was extremely high (21.5%) compared to that of dogs from individuals households (4.3%) (Itoh, *et al*, 2001). Another reports in Japan, examined rectal swaps collected from 95 dogs presented to an animal hospitals for enteric pathogens, most frequently detected in both diarrheal and normal faeces were canine corona virus (55.4%) and *Giardia lamblia* 48.2% (Mochizuki, *et al*, 2001).

Some domestic and wild animals play an important role in the transmission of giardiasis, as they may be infected with Giardia, and release a great amount of potentially infective cysts in the environment. These may be easily disseminated and contaminate water and food used for human consumption. It is therefore, very important to control infection in domestic animals and eliminate one of the main infectious sources (Jimenez-Cardoso, *et al*, 2002).

Transmission is via oral ingestion of infective cysts from faeces or contaminated food or water (Pitts, Twedit, and Malie, 1983; Stuart,*et al*, 2003).

Animals also become infected with Giardia by ingestion of contaminated materials. As pets groome themselves or one another, they can ingest cysts that were attached to the haircoat. Cysts are primarily found around the anal area (from passing infected faeces) and feet (from stepping on infected faecal matter) (Leib and Zajac, 1999).

1.7. Clinical Manifestation

Signs of giardiasis in dogs and cats range from asymptomatic carriers, to mild recurring diarrhea consisting of soft, light-colored faeces, to acute diarrhea in severe cases. Other signs associated with giardiasis are weight loss, restlessness, mucoid faeces, and poor appetite (Pitts, *et al*, 1983; Olson, *et al*, 2001). However, infected dogs do not always exhibit clinical signs for months (Itoh,*et al*, 2001; Olson, *et al*, 2001; Anderson, *et al*, 2004). Moreover, the symptoms observed vary with the life cycle stage of the parasite. Symptomatic infection occurs particulary in the bitch and its off-spring (Meyer and Jarrol, 1980). The prepatent period for Giardia is between 6 to 15 days. The incubation period may last for 12 - 19 days and marked by the first detection of the cyst in the faeces (Faubert, 2002). Giardia infection has relatively long duration particularly in adult patients where it last for at least three months (Ritchard, 1980). The prepatent period of giardiasis and the duration of infection are not related to the size of initial innoculum (Ortega and Adam, 1997). Trophozoite production progress in the small bowel until numbers reach a consistently reproducible level, at which time the host clears the infection. Mechanisms for this clearance of Giardia are unknown (Thomson, Stevens, Mahmoud, and Warren, 1976).

In human, the onset of the disease may be accompanied by nausea and low-grade fever. There may be a sudden onset of watery, foul smelling, diarrhea with flatulence and abdominal distension. Other symptoms include epigastric pain and cramping. There is an increase in fat and mucus in the stool, but no blood. Weight loss often accompanies these symptoms. The acute infection usually resolves spontaneously, although in some patients, particularly children, the acute symptoms may last for months. The acute phase is often followed by a subacute or chronic phase. Symptoms in these patients include recurrent brief episodes of loose foul stools; there may be

increased distension. Between mushy stools, the patient may have chronic continuous abdominal discomfort (Kreier and Wakelin, 1998). Factors possibly contributing to the variation in clinical manifestation include the virulence of the Giardia strain, the age of the host, and the state of the host immune system at the time of infection. Asymptomatic individuals are an important reservoir for spread of the infection (Faubert, 2000).

1.8. Diagnosis of Giardiasis

Diagnosis is confirmed by finding cysts, which may be very numerous, and in cases with diarrhea trophozoites in faecal specimens (Baker, 1973). Most laboratories rely on microscopical demonstration of the organism in faeces (Goke, Rolston, Mathan and Farthing, 1990). It is generally believed that duodenal juice will contain trophozoite even when faecal microscopy shows negative results (Craft and Nelson, 1982).

Recently, in order to improve the diagnostic tools available, immunoassays have been used to detect serum antibodies to *Giardia lamblia* (Rojas, Torres, Mediola, and Carlos, 1989). However, detection of antibodies in serum does not reflects current infection since this antibody can be circulating in patient blood for some times even after clearence of the parasite from the intestine. The availability of an immunodiagnostic assay, which can detect small amount of antigen in faeces, would have the potential to improve the diagnosis in many ways. For example ELISA have recently been developed to detect these antigens (Faubert, 2000)

Isolation of Giardia from water sources requires concentration of a large volume of water by passage through a concentrating filter at least 1 μ nominal porosity. Sample volume can range from 10 liters to hundreds of liters depending upon the source of water and the method employed. The

filter element is washed and the sediment is concentrated in the laboratory. After a series of clean-up steps, the sample concentrate is examined by the fluorescence method. The fluorescence method of detecting Giardia employs a monoclonal antibody, tagged with a fluorescent dye, which in turn seeks out the Giardia cyst in a sample concentrate. Once the antibody has located the Giardia cyst, a microscopist can examine the sample through fluorescence microscopy. The cysts are easily identified because the wall glows when exposed to the fluorescent light of the microscope (Johnson, 2000).

1.8.1. Direct Smear

It is the most common method used for diagnosis of giardiasis. However, the sensitivity of examination of trophozoites and cysts in faecal samples ranges between 30-50% (Bruke, 1975). This is probably due to the fact that cyst excretion is intermittent and the trophozoites, which are detectable only in acute cases, are very fragile and could die within hours outside their hosts. Therefore examination of multiple specimens is necessary for adequate diagnosis particularly in symptomatic cases (Pitts, *et al*, 1983; Hiatt, Markell, and Ernest, 1995).

The test procedure involves dispersion of small portion of specimen in a drop of 0.9% normal saline on a microscope slide. The sample is then covered and examined microscopically (Baker, 1973).

1.8.2. Zinc Sulphate Centrifugal Floatation

This is a concentration method for detection of Giardia cyst in faecal samples. It is probably the most accurate, practical, rapid and sensitive

diagnostic test. A zinc sulphate examination done every other day for six days identified 96% of infected dogs (Lieb and Zajac, 1999).

The test is used to detect light infection as well as others to save time by concentrating the cysts in a small volume and to eliminate the trouble caused by large faecal particles. Advantage is taken of the low specific gravity of protozoan cysts to separate them from the faeces when mixed with 33% solution of zinc sulphate (Specific gravity 1.180). The cyst will then easily be picked from the surface layer of the solution using a glass rod or bacteriologic loop and examined microscopically (Lieb and Zajac, 1999).

1.8.3. Duodenal Aspiration

Failure to find the organism in routine faecal examination does not eliminate it as a potential etiological agent of canine diarrhea. Thus, when exploratory lapratomy or endoscopy performed in-patient with consistent signs of giardiasis, an aspirate of duodenal contents should be taken and examined for Giardia spp. (Pitts, *et al*, 1983).

Negative stool findings necessitate sampling of duodenal contents either by intubations and aspiration or the use of the Entertest device described below. The device used consists of gelatin capsule inside which is packed 140 cm of white nylon thread. The patient swallows the capsule and the free end of the thread secured to the face and left in position for 4hrs. In more than 95% of cases examined by this method, the thread extends to its full length and is carried into the duodenum by peristalsis. The gelatin then dissolves and the nylon thread can be removed by gentile traction. The distal portion of the nylon which is saturated with bile stained mucus is drawn through gloved fingers; a few drops of duodenal contents are expressed onto glass slide which is examined immediately at x100

magnification to demonstrate the trophozoite (Mahmoud and Warren, 1975). This procedure is cumbersome and uncomfortable for the patient, and duodenal fluid can be negative despite other proof of infection (Unger, Yolken, Nash, and Quinn, 1984).

1.8.4. Enzyme Linked Immunsorbent Assay

Giardia antigens in faeces of infected animals have been detected with remarkable sensitivity and specificity of 98 and 100% respectively using enzyme linked immunosorbent assay (ELISA), and it was found useful in diagnosis, and in follow-up treatment (Unger, *et al*, 1984). The assay offers many advantages over other immunoassays in terms of speed, sensitivity, convenience, simplicity and reliability. The test is based essentially on two fundamental steps, the immunological reaction between the antibody and antigen and the enzymatic indicator reaction to demonstrate the presence or the absence of antibody/antigen reaction (Kurstak, 1985). Faecal ELISA kits have been developed to detect Giardia in humans and were found to be less sensitive than two or three zinc sulphate centrifugal floatation test in dogs (Barr, Bowman, and Erb, 1992; Decock, Cadiergues, Larcher, Vermot, and Franc, 2003; Anderson, *et al*, 2004).

Giardia specimens for ELISA are simple to prepare and can be stored under variety of conditions including stool-stained filter papers. The relatively long duration of antigenicity and reproducibility of result exclude the need for immediate examination of specimens. These merits give ELISA a broad applicability in epidemiological investigation in the field (Unger, *et al*, 1984).

In experimentally infected humans with Giardia cyst, Giardia antigen was found more commonly than whole cysts during the course of infection.

Most importantly, antigen was found more frequently during patency in individuals known to be infected than were Giardia cysts by routine stool examination. Therefore, during therapy even when cysts are not evident, the test may still be reliably detecting antigen, a not unexpected finding which was most likely due to the destruction of Giardia trophozoite and liberation of antigens, which is more detectable by ELISA (Nash, Herrington, and Levine, 1987).

Competitive and non-competitive ELISA can be used to detect antigens in the test samples. Competitive assays are based on the competition of the test antigen with standard amount of enzyme-labeled antigen for the immobilized antibody. The decrease in staining indicates the presence of antigen in the test sample (WHO, 1976). The non-competitive assay or double antibody sandwich method is the most popular method; however, it depends on the antigen having at least two epitopes (Tijssen, 1985). In this method the antibodies immobilized on a solid matrix followed by incubation with the test sample containing the antigen and the reaction is then detected by the addition of the primary antibody labeled with an enzyme.

1.8.5. Counter Immunoelectrophoresis (CIE)

Testing faeces extract for *Giardia lamblia* antigen by CIE appears to be specific and rapid diagnostic procedure. With refinements, including greater purity of the antigen, it could be a useful diagnostic method for clinical laboratories and a valuable research tool (Craft and Nelson, 1982).

In a study to evaluate CIE as a diagnostic method for giardiasis, in patients suffering from acute or chronic diarrhea**,** antigen was demonstrated in the faeces of all patients showing positive result by microscopy or

duodenal aspiration. Moreover, the test detected a positive case that was negative by standard diagnostic techniques. These results showed that CIE represents a diagnostic test that is as sensitive and specific as the combination of faecal and duodenal aspirate examination (Craft and Nelson, 1982).

1.8.6. Immuno Fluorescence Assay (IFA)

IFA have been used to diagnose giardiasis. Its sensitivity was found to increase when cysts were used as a source of antigen (Faubert, 2000). However, the test can detect only symptomatic patients (Rojas, *et al*, 1989; Guimaraes and Sogayar, 2002).

There are several reasons to explain the poor sensitivity of serological assays; (i) Geographical isolates have been identified, and they may have their own antigenic identity. (ii) Infection may develop into a chronic state in which the parasite may interfere with the immune system, leading to immunodepression, and this may affect the level of antibodies produced. (iii) Antigenic variation may also interfere with the production of antibodies. (iv) Many humans' cases of giardiasis never reach the acute stage of the infection. Since Giardia trophozoites rarely invade the tissue, the systemic immune response is practically never stimulated, and searching for antibodies to Giardia in the serum remains an unreliable exercise (Faubert, 2000).

1.9. Chemotherapy

From the beginning of the last century various chemicals, have been used for treatment of *Giardia lamblia* infection; these include potentially toxic substances as carbon teterachloride, mercury and bismuth (Mendelson,

1980). Limited ranges of non-toxic chemo-therapeutic are now available for treatment of Giardia infection. These comprise Metronidazole and related Nitromidazole, Quinacrine and Furazolidone. Recntely Benzimedazole group was used for treating giardiasis (Grander and Hill, 2001). However, none of them is considered to be ideal. Treatment failures are common. Repeated courses of therapy are often required and there is evidence of variable drug sensitivity between strains of Giardia (Meloni, Thompson, Reynoldson and Seville, 1990). These faitures necessitate more investigation on this aspect.

1.9.1. Quinacrine (Ataprin, Mepcrine)

After the Second World War this drug became an important agent against *Giardia lamblia*, with clinical efficacy of 90% or more. Dosing is usually 100 mg three times a day for 5 to 7 days for adults and 6mg/kg in three divided doses over 5 to 7 days for children (Grander and Hill, 2001).

The antiprotozoal mechanism of Quinacrine is not fully elucidated. The drug intercalates readily with *Giardia lamblia* DNA, and it is this interaction, which is thought to cause an inhibition of nucleic acid synthesis. In vitro, Quinacrine reduces cyst viability and excystation rate. Quinacrine is rapidly absorbed from the intestinal tract and is widely distributed in body tissues (Grander and Hill,2001).

The administration of Quinacrine is frequently accompanied by dizziness, headache, vomitting, psychotic reactions and blue or yellow staining of the skin (Meyer and Jarrol, 1980).

1.9.2. Metronidazole (Flagyl)

Metronidazole {(1-β-hydroxyethyl) -2-methyl-5-nitromidazole; Flagyl}. Is recommended for treatment of giardiasis in dogs (Olson, et *al*, 2001). Principally this drug was formulated for treatment of *Trichomonas vaginalis* and *Entamoeba histolytica* infections. Later this compound was used against giardiasis and was found to be very effective. Since then, clinicians have used Metronidazole and other Nitromidazole as the major therapy of giardiasis (Grander and Hill, 2001).

Metronidazole utilizes the anaerobic metabolic pathways present in Giardia. Trophozoites within cysts may be less affected by Mitronidazole, possibly because of poor penetration of drug through the cyst wall (Grander and Hill, 2001).

Metronidazole is quickly and completely absorbed after oral administration and penetrates body tissue and secretion such as saliva, breast milk, semen and vaginal secretions (Grander and Hill, 2001). The recommended dose for dogs is 60mg/kg of body weight daily for 5 days. Side effects are not commonly associated with administration of metronidazole. However, high doses may induce signs of neurotoxicity in dogs (Olson, *et al*, 2001), such as tremors, muscle spasms, ataxia, and even convulsions. Reversible bone marrow depression has been reported. The drug should not be used in pregnant animals, particularly during first trimester; Metronidazole rapidly enters the fetal circulation after absorption by the mother (Mendelson, 1980; Grander and Hill, 2001).

In France Metronidazole suscepitability of 11 clinical isolates of *Giardia duodenalis* was determined using a neonetal mouse model and compared with the outcome in patients after Metronidazole therapy (0.75g/day for 5 days). All isolates were clinically resistant to metronidazole. Although there were high number of Giardia metronidazole-

resistant strains, this may be explained in part by the referral of patients with therapeutic failure, suggesting the need for new antigiardial drugs (Lemee, Zaharia, Nevez, Rabodonirina, Brasseur, Ballet, and Favennec, 2000).

1.9.3. Furazolidone (Furoxone)

As early as 1950s Furazolidone was being used in the treatment of giardiasis. The mechanism of action of Furazolidone against *Giardia lamblia* is not completely explained. The drug undergoes reductive activation in the *Giardia lamblia* trophozoite, but, unlike Metronidazole, reduction possibly occurs via an NADH oxidase. Its killing effect correlates with toxicity of reduced products, which can damage important cellular components including DNA (Grander and Hill, 2001). Various studies have used regimes of 8 to 16mg/kg B.W per day for three to seven days, with curative rates ranging from 72 to 100%. Though side effects are usually mild, they occur in approximately 20% of patients (Grander and Hill, 2001) and include headache, nausea, vomitting and rashes (Mendelson, 1980).

1.9.4. Benzimidazoles

Two members of the benzimidazoles class of therapeutic, namely Albendazole (Albenz) and Mebendazole (Vermox) have been used to treat *Giardia lamblia* infection. Other benzimidazole such as Nocodazole, Oxfendazole, Thiabendazole, and Fenbendazole has also demonstrated some efficacy in humans (Grander and Hill, 2001). Benzimedazoles exerts toxic effect on Giardia in part by binding to the Giardia β-tubulin cytoskelton. This binding causes both inhibition of cytoskelton polymerization and

impaired glucose uptake (Grander and Hill, 2001). However, some of these later compound proove very efficacious in treating animal giardiasis.

Fenbendazole (Panacur) is the drug of choice for dogs; the recommended dose is 50mg/kg daily for three days (Barr, Bowman and Heller, 1994; Zajac, *et al*, 1998). It has shown to be effective in removing Giardia cyst from the faeces of infected dogs (Barr, Bowman and Heller, 1994). The drug is safe for pregnant animals as well as lactating animals. Clinical signs due to drug administration were not detected (Barr, Bowman and Heller, 1994; Zajac, *et al*, 1998).

1.9.5. Resistance

Treatment failures have been reported with all of the common anti-Giardia agents including Metronidazole, Quincrine, Furazolidine, and Albendazole. However, this may be due to reinfection from the surrounding, since it is very difficult to remove the infective cysts from the animal's environment (Olson, *et al*, 2001)

Clinically resistant strains have been treated with longer repeat courses or higher doses of original agents. However, the most efficacious means of eradicating these infections seems to involve using a drug from a different class to avoid potential cross resistance (Grander and Hill, 2001)

1.10. Control of Giardiasis

The mechanism by which the host clears *Giardia lamblia* from the intestine is not fully understood. Current evidence suggests that both humoral and cellular immune responses are important in clearing the parasite and providing immunity to reinfection (Vinayak, Kumkum and

Khana 1989; Olson, *et al*, 2001). Vinayak *et al*, (1989) suggested that the varied susceptibility to giardiasis in man might partially be attributed to the varying capacity of susceptible humans to mount antibody responses. The inability of the patient with persistent giardiasis or chronic giardiasis to clear *Giardia Lamblia* may be due to low levels of these antibodies, which appear to be essential for the killing of the trophozoites.

In addition to chemotherapy prompt removal of faeces from cages, and yards will limit environmental. Cysts are inactivated by most ammonium compounds, household bleach (1:32 or 1:16 dilution), and steam. Cysts are susceptible to desiccation and areas should be allowed to dry thoroughly after cleaning. Cysts contaminating the hair of dogs and cats may be source of reinfection. Following treatment dogs should be removed from their runs, shampooed, rinsed with disinfectant, and returned to disinfected runs (Zajac, *et al*, 1998; Payne, Ridley, Dryden, Bathgate, Milliken, Stewart, 2002). Pet's owners should be taught appropriate hygiene measures to prevent Giardia transmission from dogs to human (Itoh, *et al*, 2001).

More importantly, since drinking water is a significant source of Giardia infection, measures should be taken to treat suspected water to destroy any Giardia cysts. Such measures include boiling the water instantaneously (Meyer and Jarrol, 1980; Ortega and Adam, 1997). In drinking water cysts can be killed by 2.5% Phenol or Lysol (Mahmoud and Warren, 1975)

There are few studies on the induction of active immunity against *Giardia lamblia*. Recently, a Giardia vaccine (inactivated trophozoite) has become commercially available in the USA for prevention of clinical signs of giardiasis and reduction of cyst shedding in dogs and cats (Olson, Ceri, and Morok, 2000).

In experimental study in Mexico, the vaccine protective activity was evidenced by its prophylactic quality, as it hindered the colonization of Giardia trophozoite. It is consequently assumed that the protective effect was due to the development of the humoral capacity in vaccinated animals (Jimenez-Cardoso, Eligio-Garcia, and Cortes-Campos, 2002).

In addition, immunotherapy using Giardia vaccine has been shown to be effective in treating giardiasis in dogs. The Giardia vaccine is useful particularly in dogs that have chronic clinical signs associated with giardiasis and have not responded to treatment (Olson, *et al*, 2001). However, vaccination was not an effective treatment for asympotamitic Giardia infections (Anderson, *et al*, 2004)

CHAPTER TWO
MATERIALS AND METHODS

The present study consists of two major sections:

A survey for prevalence of giardiasis in dogs in Khartoum state and an experimental section.

2.1. The Survey

2.1.1. Survey Area

The survey covers cases brought to Police Dog Clinic from the three major twons of Khartoum state, namely Khartoum, Khartoum north and Omdurman, during the period January 2003- December 2004.

2.1.2. Animals

A total of 211 dogs of different breeds (121 males and 90 females) were surveyed. The animals were categorized into four groups as follows:

Group I 1-6 months of 115 dogs.

Group II 7-12 months of 36 dogs.

Group III 13-36 months of 49 dogs.

Group IV of over 36 months 11 dogs.

2.1.3. Examination of the Surveyed Animals

2.1.3.1. Breeds

Breeds of dogs were identified according to Fogle (1992) creteria that involves dwarf and giant dogs, noses or squashed faces, erect or lop ears, straight or curley, long or short, and black or white hair, large chest and small waist. Dog sexes and age were also recorded.

2.1.3.2. Clinical Signs

Clinical symptoms appearing on the surveyed dogs were recorded.

2.1.3.3. Direct Faecal Examination

Faecal samples were collected directly from the rectum of the surveyed dogs using a matchstick. The faecal sample was then transferred to a microscope slide and mixed with a drop of normal saline. A drop of iodine was then added to the sample. The sample was then covered with a cover slip and examined at x40 magnification using a light microscope. 20 microscopic fields were examined per each sample.

2.2. Experiment

This was carried out to evaluate the efficacy of anti-giardial drugs. Also to evaluate the diagnostic methods commonly used in diagnosis of the disease.

2.2.1. Site

The experiment was carried out in Khartoum city at the Police Dogs Adminstration of the Ministry of Internal Affairs. The dogs were kept in closed rooms with cement floor and wooden roof throughout the experiment (Figure 3). They were fed on pelleted dog meal containing all necessary

ingredients and they were watered *ad-libidum*. The kennel were immersed with Didecyldimethylammonium (Glutex. S. P. Veterinaria, s.a.Spain) for 20 mintues, rinsed with water and left to dry. This protocol was repeated daily throughout the experiment and experimental animals were bathed daily.

2.2.2. The Experimental Animals

Twenty German Shephered dogs (Figure 4) aging 6 to 24 months, weighing 17 to 36 kilograms and passing Giardia cyst in their faeces were used in this experiment. They were randomally divided into four groups of 5 dogs each: -

Group I were treated on day 1 with Metronidazole in tablet form (60 mg/kg .B.W. for 5 consecutive days)

Group II were treated on day 1 with Fenbendazole liquid (50 mg/kg B.W. for three consecutive days) followed by Metronidazole (60 mg/kg BW for 5 consecutive days)

Group III treated on day 1 with Fenbendazole (50mg/kg .B.W. for three consecutive days).

Group IV is the non-treated control.

2.2.3. Sampling

Faecal samples were collected daily for 10 ten days directly from the rectum of the experimental dogs untile the end of experiment on day 10 post treatment and examined for Giardia cyst, using both direct smear method as described earlier (section 2.1.3.3.) and zinc sulphate centrifugal floatation method described below.

Figure (3): Experimental dog's kennel.

Figure (4): Experimental Dogs (German Shepherd Dog)

Samples collected on day 1 were taken immediately before the adminstration of the drugs. Samples collected on day 1 and day 10 were also preserved at -20°C for ELISA examination.

2.2.4. Zinc Sulphate Centrifugal Floatation

Two grams of faeces were mixed with 15 ml of 33% zinc sulphate solution and then strained through a 1 mm mesh sieve. A centrifuge tube (15 ml volume) was filled with this strained mixture and centrifuged 3 to 5 minutes at 1500 rpm. The surface layer was transferred to a microscope slide with a bacteriologic loop and a drop of 1% iodine was added. The sample was then covered with a cover slip and examined at x40 using a light microscope.

2.2.5. Enzyme Linked Immunosorbent Assay (ELISA)

This assay was used to detect Giardia antigens in the faeces of experimental animals before and after treatment using Novatec ELISA kit (Novatec, Germany).

2.2.5.1. ELISA Kit Reagents and Buffers

1- *Giardia lamblia* antibody coated wells: 12 break apart 8-well snap-off strips coated with polyclonal anti-*Giardia lamblia* antibodies.

2- Sample Diluent: 1 bottle containing 100 ml of ready to use buffer (PBS) for sample dilution; pH8.4± 0.1.

3- Washing Solution (10x conc.): 1 bottle containing 100 ml of a 10 fold concentrated buffer for washing the wells; pH 7.4± 0.1 (PBS).

4- Stop Solution: 1 bottle containing 15 ml sulpharic acid, 0.25

mol/L; ready to use.

5- Tetramethylbenzidine (TMB) substrate solution: 1 bottle containing 15 ml 3,3,5,5-

6- Anti-*Giardia lamblia* Conjugate: 1 bottle containing 12 ml of peroxidase labeled anti-*Giardia lamblia* antibodies; ready to use.

7- *Giardia lamblia* Antigen Positive Control: 1 bottle containing 2 ml antigen; ready to use.

8- *Giardia lamblia* Antigen Negative Control: 1 bottle containing 2 ml; ready to use.

2.2.5.2. ELISA Procedure

Following manufacturer instructions the test samples were diluted 1:11 with sample diluent.A 100µl alquots of each sample were dispensed in duplicates into the appropriate wells of the antibody coated microtitre strips. Duplicates of positive and negative controls and substrate blanks wells were also included in the test run (Figure 5)

The wells were then covered with foil and incubated for 30 minutes at room temperature. Unbound antigens were removed by aspirating the contents of the wells and washing with 5x5 seconds washing cycles with washing solution. The remaining fluid was eventually removed by tapping strips on tissue paper. Then 100µl aliquots of a peroxidase labeled anti- *Giardia lamblia* antibodies were dispensed into each well except for the blank wells. The wells were again covered with foil and incubated for 30 minutes at room temperature. Unbound conjugate

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	**SB**	2	3	4	5	6	7	8	9	10	11	12
B	**SB**	2	3	4	5	6	7	8	9	10	11	12
C	**NC**											
D	**NC**											
E	**PC**											
F	**PC**											
G	1						40					
H	1						40					

Figure (5): ELISA plate's layout

SB: Substrate Blank
NC: Negative Control
PC: Positive Control
1-40 inside squares denotes sample numbers

was then removed by washing with washing solution as described above. A ready to use 3,3,5,5 teteramethylbenzidine (TMB) solutions was added in 100µl aliquots to all wells and incubated in the dark for 10 minutes at room temperature. The reaction was then stopped by adding 100µl of 2.5 mol/L sulphuric acids to each well and the absorbance of each well was then measured photometrically at 450 nm using an ELISA plate reader photometer adjusted to zero using the blank wells.

2.2.5.3. Cut-off Determination

The cut-off point was calculated according to the manufacturer instructions by addition 0.20 absorbance units to the measured absorption of the mean value of the two negative control wells.

Cut-off = Mean absorbance value of the negative controls + 0.20

Samples were considered positive if the absorbance value is higher than 10% over the cut-off, while those with absorbance values lower than 10% over the cut-off point were considered negative.

2.3. Statistical analysis

For the analysis of data collected from experimental results a statistical analysis system (SAS) package was used. The SAS system was used to perform Kappa coefficient and T test.

CHAPTER THREE

REUSLTS

3.1. Survey of Giardia Infection in Dogs in Khartoum State:

3.1.1. Dog Breeds Surveyed:

Twelve breeds of dogs were examined for giardiasis in the present study including German shepherd, Local breed, Mastiff, Retriever, Saluki, Doberman, Albi, Terierr, St Bernard, Rhodesia Ridgeback, Dalmatian and Boxer.

3.1.2. Breed Susceptibility

As shown in table (1) Giardia cysts were detected in almost all breeds of dogs surveyed were positive. The overall prevalence being 70.6%.

3.1.3. Prevalence of Giardia in Different Locations in Khartoum State:

Using direct faecal examination Giardia cysts were detected in faecal samples of 164 dogs (69.1%) out of 211 dogs surveyed in Khartoum State.

The disease was detected in dogs examined in the three major zones of Khartoum state. Table (2) shows the prevalence of infection in these Locations. 66.7% was reported in Khartoum North, 67.7% in Khartoum, and 82.6% was reported in Omdurman.

Table 1: Prevalence Rates of *Giardia Lamblia* in Different Breeds of Dogs in Khartoum State During (January 2003 – January 2004).

Breed	No. examined	Positive
German Shepherd dog	163	117 (77.8%)
Local breed	16	12 (75%)
Retriever	8	5 (62.5%)
Terrier	7	3 (42.9%)
Total	194	137 (70.6%)

Table 2: Prevalence Rates of *Giardia lamblia* in Dogs in Different Locations in Khartoum State During January 2003 – January 2004.

Location	No. examined	Positive
Khartoum	155	105(67.7%)
Khartoum North	33	22 (66.7%)
Omdurman	23	19(82.6%)
Total	211	146(69.1%)

3.1.4. Seasonality of Infection:

As shown in table (3) the disease was prevalent throughout the year. The prevalence rate being 71.9% in winter, 68.8% in summer and 68.8% in autumn.

3.1.5. Age Groups Susceptibility:

Table (4) shows the prevalence rate of Giardia among different age groups. The parasite infects all ages. A prevalence of 72.2% was detected among those between 1-6 months old, 72.2% in dogs between 7-12 months, 61.3% among those between 13-36 months and 63.7% among dogs over 36 months old.

3.1.6. Prevalence of Giardia in Different Sexes:

As shown in Table (5) the parasite can infect both sexes with a prevalence rate of 72.7% in males and 64.4% in females.

Table 3: Prevalence Rates of *Giardia lamblia* in Dogs in Different Seasons in Khartoum State During the Period Jaunary 2003 – January 2004.

Season	No. examined	Positive
Winter	96	69 (71.9%)
Summer	80	55 (68.8%)
Autumn	32	22 (68.8%)
Total	211	146 (69.1%)

Table 4: Prevalence Rates of *Giardia lamblia* in Different Age Groups of Dogs in Khartoum State During January 2003 – January 2004.

Age group	No. examined	Prevalence
1-6 months	115	83 (72.2%)
7-12 months	36	26 (72.2%)
13-36 months	49	30 (61.3%)
>36 months	11	7 (63.7%)
Total	211	146(69.1%)

Table 5: Prevalence Rates of *Giardia Lamblia* in Male and Female Dogs in Khartoum State During January 2003 – Jaunary 2004.

Sex	No. examined	Positive
Male	121	88(72.7%)
Female	90	58(64.4%)
Total	211	146(69.1%)

3. 1.7. Risk Factor Analysis

The mean of positive cases in Khartoum (3.1±1.7) and the mean of negative cases (5.0±4.5) (P>0.05) (Table 6). The mean of positive cases in Khartoum North (1.1±0.3) and the mean of the negative cases (2.2±1.6) (P<0.05). The mean of positive cases in Omdurman (1.0±0.0) and the mean of negative cases (1.9±1.1) (P<0.05) (Table 6).

The mean of positive cases in winter (4.3±4.5) and the mean of negative cases (2.3±1.8) (P>0.05). The mean of positive cases in summer (3.9±3.4) and the mean of negative cases (2.2±1.6) (P>0.05). The mean of positive cases in autmn (2.0±1.3) and the mean of negative cases (1.7±1.6) (P>0.05) (Table 7).

The mean of positive cases in dogs between 1-6 months (4.9±4.9) and the mean of negative cases (2.7±2.1) (P>0.05). The mean of positive cases in dogs between 7-12 months (2.3±1.5) and the mean of negative cases (1.7±1.6) (P>0.05). The mean of positive cases in dogs between 13-36 months (3.6±2.7) and the mean of negative cases (2.2±1.3) (P>0.05). The mean of positive cases in dogs over 36 months (1.6±8.9) and the mean of negative cases (1.0±0.0) (P>0.05) (Table 8).

The mean of positive cases in male (3.8±4.4) and the mean of negative cases (2.2±1.7). The mean of positive cases in females (2.1±1.5) and the mean of negative cases (3.2±2.3) (P>0.05) (Table 9).

3.3. Experimental Results

Samples collected from experimental animals before treatment were all positive when examined by both direct smear and zinc sulphate centrifugal floatation test.

Table 6:Risk factor of locality

Sor.V	N	Means ± Std	P
Locality			
Khartoum			
Negative	16	5.000 ± 4.494	0.092
Positive	21	3.125 ± 1.746	
Khartoum North			
Negative	10	2.200 ± 1.619	0.049
Positive	10	1.100 ± 0.316	
Omdurman			
Negative	4	1.900 ± 1.100	0.029
Positive	10	1.000 ± 0.000	

P significant ≤0.05

Table 7: Risk factor for season

Sor.V	N	Means± Std	P
Season			
Winter			
Negative	13	2.307 ±1.750	0.123
Positive	16	4.312 ± 4.585	
Summer			
Negative	11	2.272 ±1.618	0.132
Positive		3.812 ± 3.432	
Autumn			
Negative	6	1.666 ± 1.618	0.675
Positive	11	2.000 ±1.264	

P significant ≤0.05

Table 8: Risk factor for age

Sor.V	N	Means± Std	P
Age Groups			
1-6 months			
Negative	12	2.666 ±2.103	0.111
Positive	17	4.882 ± 4.922	
7-12 months			
Negative	6	1.666 ± 1.211	0.318
Positive	11	2.363 ±1.501	
13-36months			
Negative	9	2.222 ±1.301	0.207
Positive	8	3.625 ± 2.625	
Over 36 months			
Negative	3	1.000 ± .000	0.208
Positive	5	1.600 ± 8.944	
P significant ≤0.05			

Table 9: Risk factor for sex

Sor.V	N	Means± Std	P
Sex			
Male			
Negative	15	2.200± 1.740	0.124
Positive	23	3.826±4.437	
Female			
Negative	15	3.222±2.315	0.121
Positive	18	2.133±2.315	
P significant≤ 0.05			

3.2.1. Treated Groups:

3.2.1.1. Metronidazole Group

Following treatment with Metronidazole, Giardia cysts persist in faeces of dogs of this group throughout the length of the experiment, the cyst disapeared from 4 dogs on day 2, and appear in 4 dogs on day 3, on day 4 and day 6 it disappeared from faeces of only 3 dogs and on day 9 from 4 dogs, and from 2 dogs on day 5. On day 10 the cyst was detected in the 5 dogs (Figure 6). In the control group, cysts disappeared from the faeces of 4 dogs on day 3 of experiment and from all dogs of this group on day 4. Thereafter, the cysts were detected in the faeces of all dogs except on day 9 and 10 when they disappeared from the faeces of only 2 dogs.

3.2.1.2. Fenbendazole Group

Following treatment with fenbendazole, Giardia cysts in dogs of this group disappeared from faeces on day 2 from 4 of the treated dogs. While on day 3 cysts appeared again in faeces of 4 dogs and in 2 dogs on day 4and day 5. Thereafter the cysts disappeared from the faeces of all treated dogs until the end of experiment on day 10 except on day 7 when cysts appeared in all dogs (Figure, 6)

3.2.1.3 Combined Metronidazole and Fenbendazole Group

Following the combined treatment with the two drugs, Giardia cysts were not detected in the faeces of 4 of the dogs in this group on day 2, cyst appeared in 4 dogs on day 3, and disappeared from faeces of all dogs on days 4,6 and 9 and from the faeces of only 2 dogs on day 10, the cyst appear in 4 dogs on day 7,8. (Figure 6).

3.2.2. Comparison Between Parasitological and Immunological Findings:

The cross tabulation between direct smear test and zinc sulphate centrifugal floatation test results revealed that 26 samples were agreed upon by the two tests. A total of 26 (100%) positive samples were detected by direct smear and 31(83.9%) by zinc sulphate centrifugal floatation test (Table 10). Also the two tests agreed on 9 negative samples, out of 14 (64.3%) negative samples by direct smear (Table 10). On the other hand, the two tests were disagreed on 5 samples, which were positive by ZSCFT and negative by direct smear (Table 10).

The cross tabulation between direct smear and ELISA results showed that the two tests agreed on 22 positive samples, out of 26 (84.6%) positive samples detected by direct smear and 26 (84.6%) positive samples using ELISA (Table 11). Also 10 negative samples were agreed upon by the two tests out of 14 (71.4%) negative samples detected by direct smear and 14 (71.4%) negative samples by ELISA (Table 11). On the other hand, the two tests disagreed on 4 samples, which were positive by ELISA but negative by direct smear. Moreover, there were 4 samples positive by direct smear but negative by ELISA (Table 11).

The cross tabulation between zinc sulphate centrifugal floatation test and ELISA results showed that the two tests agreed on 23 positive samples, out of 31(74.2%) positive samples detected by zinc sulphate centrifugal floatation test and 26 (88.5%) positive samples using ELISA (Table 12). Also 6 negative samples were agreed upon by the two tests, out of 9 (66.7%) negative samples detected by zinc sulphate centrifugal floatation test and 14 (42.9%) negative samples by ELISA

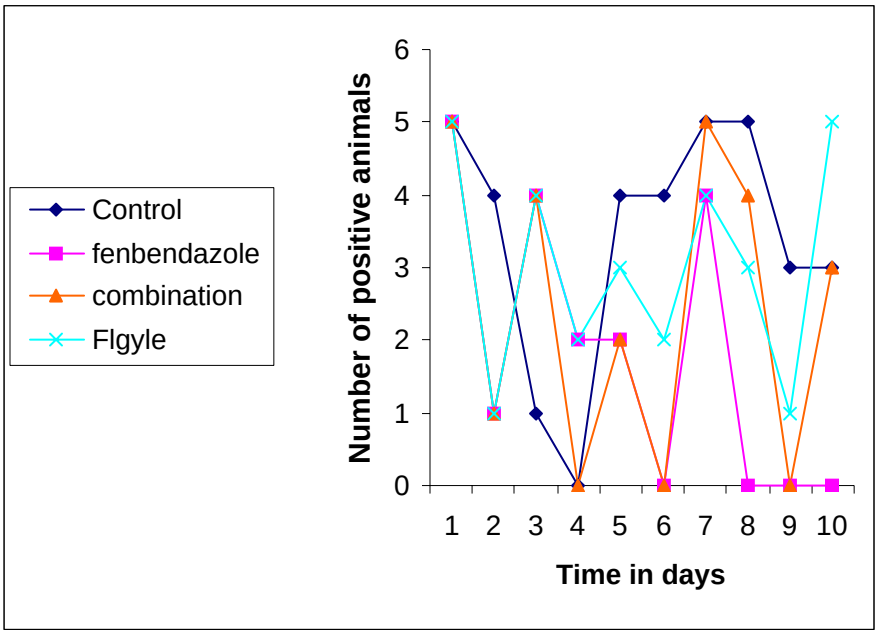

Figure (6): Effect of chemoyherapy on Giardia cyst production in experimental animal.

Table 10:Cross Tabulation Between Results Obtained From Direct Smear test and Zinc Sulphate Centrifugal Floatation Test.

	Direct smear		
ZSCFT	Negative	Positive	Total
Negative	9	0	9
Positive	5	26	31
Total	14	26	40

Table 11:Cross Tabulation Between Results Obtained From Direct Smear Test and ELISA Test.

	Direct smear		
ELISA	Negative	Positive	Total
Negative	10	04	14
Positive	04	22	26
Total	14	26	40

(Table 12). On the other hand, the two tests disagreed on 8 samples, which were positive by zinc sulphate centrifugal floatation test but negative by ELISA. Moreover, there were three samples positive by ELISA and negative by ZSCFT (Table 12).

The level of agreement between direct smear and zinc sulphate centrifugal floatation test was found to be high (85%) with Kappa coefficient value of 0.628 (P = 0.000) (Table10). That between direct smear and ELISA was also found to be high (77.5%) with Kappa coefficient value of 0.497 (P= 0.000) (Table 10). The same picture was obtained when the level of agreement between zinc sulphate centrifugal floatation test and ELISA was tested, being 72.5% with Kappa coefficient value of 0.341(P= 0.03) (Table 13).

Table 12: Cross Tabulation Between Results Obtained From ELISA and ZSCFT test

	ZSCFT		
ELISA	Negative	Positive	Total
Negative	6	8	14
Positive	3	23	26
Total	09	31	40

Table 13: Levels of Agreement Between Different Diagnostic Methods

	Agreement	Kappa	P
Direct smear x ZSCF	85.00%	0.628	0.000
Direct smear x ELISA	77.5%	0.492	0.000
ZSCFT x ELISA	72.5%	0.341	0.013

CHAPTER FOUR
DISCUSSION

Although Giardia is capable of infecting a wide range of hosts, and has been detected in a wide range of mammals, including humans and dogs. The role of dogs in transmission of infection to man is obscure and yet to be identified (Leib and Zajac, 1999; Itoh, *et al,* 2001; Mochizuki, *et al*, 2001; Faubert, 2000; Keulen, *et al,* 2002; Staurt, *et al,*2003). In the sudan, the prevalence of the disease in humans in Khartoum State was estimated at 18.2% during the year 2003 (Ministray of Health 2004). However, no records of the disease in dogs was available although dog ownership is increasing among residents for security reasons.

In the present study, however, dogs were found very suscpetible to infection and an overall prevalence of 70.6% was detected among them in Khartoum city using direct faecal examination. Moreover, all breeds of the surveyed dogs were found suscpetible to infection necessitating investigation in thier role as a source of infection to human. These results agreed with those of Diaz *et, al,* (1987), Rahman, (1990), Nikolic, *et al*, (1992), Grimason, *et al,*(1993) Johnston, and Gasser (1993), Khalifa, (1999), Hansen, *et al,* (2000), Itoh, *et al,* (2001) and, Mochizuki, *et al,* (2001) in different parts of the world.

Within Khartoum state, giardiasis was detected in dogs in the three major towns with high significant between positive and negative cases in Omdurman and Khartoum North, possibly because dogs in these locations are raised for trade purposes and are caged together rendering it easy for the parasite to spread among them. Unlike in Khartoum state where these animals are raised as guard dogs, kept separately and taken good care of.

In this study sex was not found to have an effect on suceptability of dogs to infection; both sexes were found suceptible with a prevalence of 72.7% in males and 64.4% in females and no significant between positive and negative cases in the two sex.

Although all age groups surveyed were found susceptible to the infection and significant between positive and negative cases in all groups, dogs less than one year showed high prevalence rate compared to other age groups . Possibly because dogs in this age are usually kept together indoors. Similar findings were reported by Diaz, *et al*, (1987), Nickolic, *et al*, (1992), Hansen, *et al*, (2000), Itoh, *et al* (2001), Mochizuki,*et al*, (2001), and Anderson, *et al*, (2004) in different parts of the world.

Concerning seasonality of infection, the disease was found to prevail throughout the year in Khartoum state without difference between different seasons because there was no significant between positive and negative cases in the three seasons. This is possibly because microclimate is suitable for the survival of the parasitic cysts which can survive for months in moist habitat (Meyer, Jarrol, 1980). The persistence of infection throughout the summer and autumn is also in agreement with the findings of Itoh, *et al*, (2005) who examined Giardia cysts in sewage to estimate giardiasis prevalence among inhibtants in Japan.

Dogs showing typical symptoms of giardiasis, or other suffering from unrelated illness and those who looked appearently healthy, were all found to shed cysts in their faeces in the present study. Likewise Itoh, *et al*, (2001) were able to detect Giardia cysts in faeces of apperently healthy dogs, and Anderson, *et al*, (2001) who examined dogs for giardiasis at a Canadian veterinary clinic reported that 78% of positive cases were asymptomatic. They attributed these findings to the fact that the disease can be

asymptomatic in certain phases of infection and such dogs are supposed to be carrier of the disease and play a major role in the contamination of the environment. These findings necessitate the routine examination of domestic dogs in order to reduce the contamination risk.

The traditional parasitological techniques such as direct faecal examination and zinc sulphate centrifugal floatation test are the common diagnostic techniques for giardiasis. Direct faecal examination is the routine test for both human and animal cases. Although the test can detect both cyst and trophozoite and is simple and easy to perform (Goka, *et al*,1990) its sensitivity is low ranging from 30%- 50% (Pitts, *et al*, 1983; Hiatt, *et al*, 1995).

On the other hand zinc sulphate centrifugal floatation test; a sample concenteration test, is more sensitive and considered to be the gold standared test for detection of giardiasis in dogs (Barr, Bowman and Heller, 1994; Zajac, *et al*, 1998; Anderson, *et al*, 2001, and Olson, *et al*, 2001). More recently the immunologaical assay ELISA have been used to detect Giardia antigen in faeces of infected animals and was found to be more releable as a screening test particulary when large number of samples have to be examined (Anderson, *et al*, 2001).

Of a 20 positive samples examined by direct faecal examination and zinc sulphate centrifugal floatation test at the begining of the present experiment 2 were found negative when examined by ELISA, indicating that a false negative ELISA does not rule out infection. In the other 20 samples taken from the experiment dogs after treatment at the end of the experiment, Giardia cyst was detected in 6 samples when examined by direct smear and 11 samples when examined by zinc sulphate centrifugal floatation while Giardia antigen was detected in 8 samples when examined by ELISA. These

finding does not necessairly indicate that ELISA is less sensitive than zinc sulphate centrifugal floatation since the test detects that only antigens liberating from destruction of trophozoites after chemotherapy and their availability is dependent on the efficacy of the chemotherapeutic agent.

These resulst agreed with Decock, *et al*, (2003) who suggested that if a single zinc sulphate centrifugal floatation is insufficient, two or three zinc sulphate centerifugal floatation and one or two ELISA have almost the same sensitivity. The result also agreed with Masami,et, al, (2001) who examined 95 dogs presented at an animal hospital in Japan and found 20.6% of them positive by zinc sulphate centrifugal floatation test and 48.2% were positive by ELISA test.

Kappa cofficient show high agreement between direct faecal examination and zinc sulphate centrifugal floatation test (K = 0.64), this may be due to the fact that the experimental dogs were selected on the basis of positivity by direct smear and hence at least 50% of the samples under consideration were agreed upon from the beginning of the experiment.

The disagreement between ELISA and zinc sulphate centrifugal floatation (Kappa = 0.34) in the present study was not unexpected since the liberation of antigens is dependable upon the efficacy of the drug.

In diagnosis Giardia infection and/or evaluating antigiardial drugs through detection of shedded cysts in the faeces of infected animals, zinc sulphate centrifugal floatation proved to be the most suitable test in the present study. The test offers many advantages over the other two tests in terms of sensitivity, convenience, simplicity and cheap. On the other hand, however, ELISA can be suitable for epidemiological study as screening test particularly when large number of samples have to examine.

In the present study asymptomatic dogs have been shown to shed cysts in their faeces, necessitating routine treatment of dogs showing symptoms of giardiasis and asymptomatic dogs since the latter animals may play an important role in the transmission of giardiasis, by releasing a great amount of potentially infective cysts in the invronment (Jimenez-Cardoso, *et al*, 2002).

Certain chemicals have been used for treatment of giardiasis. metronidazole, which utilizes the anaerobic pathways in Giardia, is the most common drug used for human and dogs in Sudan. However, certain strains of Giardia have developed resistant to this drug. In France metronidazole suscepitabilty of 11 clinical isolates of *Giardia duodenalis* was determined using a neonetal mouse model and compared with the outcome in patients after standared metronidazole therapy (0.75g/day for 5 days). All isolates were found to be clinically resistant to metronidazole, suggesting the need for new antigiardial drugs (Lemee, *et al*, 2000). Redy, Rai, Ranganath, Chandrashekarmurthy, Nagarajachar, (1992), used metronidazole for treatment of giardiasis in 20 dogs examined by faecal examination. Full remission of clinical signs was achieved within 3 days in 12 of the dogs and in 5 days in the remaining 8 dogs. The recommended dose is 60mg/kg for 5 days. Trophozoites within cysts may be less affected by mitromidazoles (Timothy and David, 2001). Likewise in the present study dogs in the group treated with metronidazole were shedding Giardia cyst until the last day of the experiment when zinc sulphate centrifugal floatation test was used as screening test. Suggesting the presence of resistant strains or possibly because this drug does not penetrate the cyst wall (Grander and Hill, 2001, Lemee, *et al*, 2000).

Recently fenbedazole have been tried to treat canine giardiasis and was found to be very effective against this infection. The drug exerts toxic effect on Giardia by binding to the Giardia β-tubulin cytoskelton. This binding causes both inhibition of cytoskelton polymerization and impaired glucose uptake (Grander and Hill, 2001). The drug was also found to decrease sheding of Giardia cysts in cats to less than detectable numbers (Keith, Radecki, and Lappin, 2003). The recommended dose is 50 mg/kg for three days (Barr and Heller, 1994; Zajac, *et al*, 1998; Anderson, *et al*, 2001, and Olson, *et al*, 2001). In the present study the group of dogs treated with fenbedazole stop shedding cyst in faeces, Giardia antigen was also detected in these dogs indicating the destruction of trophozoites, this result agreed with that of Barr and Heller, (1994), Zajac, *et al*, (1998), Anderson, *et al*, (2001), and Olson, *et al*, (2001).

The efficacy of the combined therapy was not high in the present study. 40% of the treated dogs in this group were shedding cyst in their faeces throughout the cource of the experiment.

CONCULSION AND RECOMMENDATIONS

The present study concluded that canine giardiasis is widely distributed in Khartoum with higher prevalence rates beining in Omdurman followed by Khartoum North and Khartoum. However, more detailed study is needed in each location in order to monitor the disease situation in stray dogs which were not included in the present study.

The study also demonstrated that there are a number of risk factors, which influence the epidemioloy of the disease. On the other hand, the study revealed that there is no strong evidence that host sex or breed play a major role in the epidemiology of canine giardiasis.

In this study also symptomatic and asymptomatic dogs examined were found to shed Giardia cysts in their faeces necessitating a routine examination and treatment of all dogs in order to reduce environmental contamination. However, a more detailed study is needed to determine the incidence of the disease among these appearantly healthy dogs and their role in spreeding the infection.

Reduction of environmental risk will also necessitate a survey study for monitoring Giardia cysts in drinking water.

Assessment of diagnostic methods commonly used for diagnosis of Giardia infection revealed that zinc sulphate centrifugal floatation is most suitable for diagnosis canine giardiasis and monitoring successful chemotherapy. Zinc sulphate centrifugal floatation is cheaper than the ELISA reagents, easy to perform and doesn't require sophisticated equipments. The ELISA however, is more suitable in epidemiological studies as screening test when large number of samples have to be examined

Evaluation of chemotherapeutic agents for treatment of canine giardiasis revealed that fenbendazole is the drug of choice in reducing the number of cysts in infected dogs. However, further investigations are needed in search for an effective therapy for canine giardiasis.

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ملخص الأطروحة

مرض الفاردیا منتشر في جمیع أنحاء العالم بصورة واسعة في الإنسان والحیوان ولكن لا توجد معلومات عنه في السودان مع إن المرض یمثل مشكلة لأصحاب الكلاب.

أجریت هذه الدراسة لمعرفة مستوى وجود المرض في ولایة الخرطوم ومقارنة ثلاثة طرق مستخدمة للتشخیص وإیجاد دواء مناسب لعلاج المرض.

تم فحص ٢١١ كلب في عیادة الكلاب البولیسیة بالخرطوم بواسطة المسحة المباشرة ووجد الطور المتكیس في ١٤٦ من الكلاب التي تم فحصها (٧٠,٦%). تم تسجیل نسبة (٨٢,٦%) في أمدرمان و (٦٧,٧%) فى الخرطوم و (٦٦,٨%) في الخرطوم بحري.كانت الإصابة بنسب عالیة في كل السلالات التي فحصت. تم تسجیل (٧١,٩%) في فصل الشتاء، وكانت المعدلات متساویة في فصلى الصیف والخریف

(٦٨,٨%). تم تسجيل (٧٢,٧%) في الذكور و (٦٤,٤%) في الإناث. تم تقسيم الكلاب إلى ٤ فئات حسب أعمارها و تم تسجيل (٧٧,٢%) في الفئة العمرية الأولى ما دون ٦ شهور كأعلى نسبة و (٦٢,٣%) كأقل نسبة في الفئة العمرية بين سنة وثلاثة سنوات.

تم أجراء تجربة لمقارنة الاختبارات الثلاثة التي تستخدم لتشخيص المرض المسحة المباشرة- اختبار الطفـو والترسيب بكبريتات الزنك - اختبار الاليزا. استخدم ٢٠ كلب. وأخذت منها عينات في ٢٠ عينه في اليوم الأول وكانت كلها موجبة باختباري المسحة المباشرة و اختبار الطفو والترسيب بكبريتات الزنك وجد الطور المتكيس في عينا ا و٩٠% من هذه العينات كانت موجبة عند استخدام اختبارالاليزا. أخذت ٢٠ عينـة في اليـوم الأخـير وكانت ٣٠% منها موجبة لاختبار المسحة المباشرة و٤٠% موجبة عند استخدام اختبار الاليزا او٥٥% لاختبار الطفو والترسيب بكبريتات الزنك.

تم تقسيم هذه الكلاب لأربعه مجموعات في كل مجموعة ٥ كلاب واستخدم الميترونيدازول والفينبيندازول والاثنين معا. ا موعة التي تم علاجها بالميترونيدازول ٦٠ملجم /كجم من وزن الجسم لمدة ٥ أيام وجـد الطـور المتكيس في كل ا موعة وظهور الانتجين في ٢ من هذه ا موعة في اليوم العاشر من بداية التجربة. ا موعة (٢) تم علاجها بالفينبيندازول ٥٠ ملجم/كجم من وزن الجسم لمدة ثلاثة أيام ثم بالميترونيدازول ٦٠ملجم /كجم من وزن الجسم لمدة ٥ أيام وجد الطور المتكيس في ٣ من هذه ا موعة وظهور الانتجين في واحد من هذه ا موعـة. (٣) عولجت بالفينبيندازول ٥٠ ملجم/كجم من وزن الجسم لمدة ثلاثة أيام في اليوم العاشر ظهر الانتجين في واحد مـن

هذه المجموعة. المجموعة التي لم تعالج وجد الطور المتكيس في ٣ من هذه المجموعة وظهور الانتجين في ٣ من هذه المجموعة.

استخدام اختبار الطفو والترسيب بكبريتات الزنك اقتصادي ويسهل التعرف على الطور المتكيس وسريع ورخيص. الفينبيندازول فعال في إيقاف إخراج الطور المتكيس.